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(71) Applicant: PRESIDENT AND FELLOWS OF HARVARD COLLEGE [US/US]; 17 Quincy Street, Cambridge, MA 02138 (US).

(72) Inventors: URBAN, Robert, Glen ; 76 Hatfield Road, Newton, MA 02165 (US). CHICZ, Roman, M. ; 82 Glen Road, Jamaica Plain, MA (US). VIGNALI, Dario, A., A. ; 761 Lower Rainham Road, Kent ME8 7UB (GB). HEDLEY, Mary, Lynne ; 214 Walden Street, #1, Cambridge, MA 02140 (US). STERN, Lawrence, J. ; 181 Newport Street, Arlington, MA 02174 (US). STROMINGER, Jack, L. ; 2030 Massachusetts Avenue, Lexington, MA 02173 (US).

(74) Agent: CLARK, Paul, T.; Fish & Richardson, 225 Franklin Street, Boston, MA 02110-2804 (US).

(54) Title: IMMUNOMODULATORY PEPTIDES

(57) Abstract

A purified preparation of a peptide comprising an amino acid sequence identical to that of a segment of a naturally- occurring human protein, said segment being of 10 to 30 residues in length, inclusive, wherein said peptide binds to a human major histocompatibility complex (MHC) class II allotype.

- 1 -

IMMUNOMODULATORY PEPTIDES

The field of the invention is major
histocompatibility complex (MHC) antigens.

5   Background of the Invention

Major histocompatibility complex (MHC) class II
antigens are cell surface receptors that orchestrate all
specific immune responses in vertebrates. Humans possess
three distinct MHC class II isotypes: DR, for which
10 approximately 70 different allotypes are known; DQ, for
which 33 different allotypes are known; and DP, for which
47 different allotypes are known. Each individual bears
two to four DR alleles, two DQ alleles, and two DP
alleles.

15   MHC receptors (both class I and class II)
participate in the obligate first step of immune
recognition by binding small protein fragments (peptides)
derived from pathogens or other non-host sources, and
presenting these peptides to the regulatory cells (T
20 cells) of the immune system. In the absence of MHC
presentation, T cells are incapable of recognizing
pathogenic material. Cells that express MHC class II
receptors are termed antigen presenting cells (APC).
APCs ingest pathogenic organisms and other foreign
25 materials by enveloping them in endosomic vesicles, then
subjecting them to enzymatic and chemical degradation.
Foreign proteins which are ingested by APCs are partially
degraded or "processed" to yield a mixture of peptides,
some of which are bound by MHC class II molecules that
30 are en route to the surface. Once on the cell surface,
MHC-bound peptides are available for T cell recognition.

MHC class II antigens are expressed on the surface
of APCs as a trimolecular complex composed of an α chain,
a β chain, and a processed peptide. Like most
35 p lypeptides that are expressed on the cell surface, both

- 2 -

α and β chains contain short signal sequences at their NH_2 termini which targ t them to the endoplasmic reticulum (ER). Within the ER the class II α/β chain complex associates with an additional protein termed the

5 invariant chain (Ii). Association with Ii is proposed to block the premature acquisition of peptides (by blocking the peptide binding cleft of the MHC heterodimer), promote stable α/β interaction, and direct subsequent intracellular trafficking of the complex to endosomal

10 vesicles. In the endosomes, Ii is removed by a process involving proteolysis; this exposes the peptide binding cleft, thus allowing peptides present in the endosome to bind to the MHC molecule. The class II/ peptide complex is transported from the endosomes to the cell surface

15 where it becomes accessible to T-cell recognition and subsequent activation of immune responses. Class II MHC molecules bind not only to peptides derived from exogenous (ingested) proteins, but also to those produced by degradation of endogenous (self) proteins. The amount

20 of each species of peptide which binds class II is determined by its local concentration and its relative binding affinity for the given class II binding groove, with the various allotypes displaying different peptide-binding specificities.

25   Early during fetal development, the mammalian immune system is "tolerized", or taught not to react, to self-peptides. The stability and maintenance of this system is critical for ensuring that an animal does not generate an immune response against self. A breakdown of

30 this system gives rise to autoimmune conditions such as diabetes, rheumatoid arthritis and multiple sclerosis. Current technologies intended to manipulate the immune system into reestablishing proper nonresponsiveness include pr toc ls inv lving th intravenous deliv ry of

35 synthetic, high affinity binding peptides as blocking peptides.

- 3 -

Vaccination can generate protectiv immunity against a pathogenic organism by stimulating an antibody-mediated and/or a T cell-mediated response. Most of the current vaccination strategies still use relatively crude
5 preparations, such as attenuated or inactivated viruses. These vaccines often generate both antibody- and cell-mediated immunity, and do not allow one to modulate the type of immune response generated. Moreover, in many diseases the generation of the wrong type of response can
10 result in an exacerbated disease state.

Summary of the Invention

In the work disclosed herein, naturally processed peptides bound to six of the some 70 known human MHC class II DR allotypes (HLA-DR1, HLA-DR2, HLA-DR3, HLA-
15 DR4, HLA-DR7, and HLA-DR8) have been characterized. These peptides were found to be predominantly derived from self proteins rather than foreign proteins. Several self peptide families have been identified with the unexpected property of degenerate binding: that is, a
20 given self-peptide will bind to a number of HLA-DR allotypes. This observation runs counter to the widely-accepted view of MHC class II function, which dictates that each allotype binds a different set of peptides. Furthermore, many if not all of the self-peptides
25 disclosed herein bind to the class II molecules with relatively high affinity. These three characteristics--(1) self rather than foreign, (2) degeneracy, and (3) high affinity binding--suggest a novel means for therapeutic intervention in disease conditions
30 characterized by autoreactivity, such as Type I diabetes, rheumatoid arthritis, and multiple sclerosis. In addition, such therapy could b used to reduce transplant rejection.

- 4 -

In the therapeutic methods of the invention, short peptides modelled on the high-affinity immunomodulating self peptides of the invention (which preferably are nonallelically restricted) are introduced into the APCs

5 of a patient. Tissue typing to determine the particular class II alleles expressed by the patient may be unnecessary, as the peptides of the invention are bound by multiple class II isotypes. It may be useful to employ a "cocktail" of peptides, where complete

10 degeneracy is lacking for individual peptides, i.e., where peptides binds to fewer than all allotypes; the cocktail provides overlapping binding specificity. Once in the APC, a peptide binds to the class II molecules with high affinity, thereby blocking the binding of

15 immunogenic peptides which are responsible for the immun reaction characteristic of the disease condition. Because the blocking peptides of the invention are self peptides with the exact carboxy and amino termini tolerized during ontogeny, they are immunologically inert

20 and will not induce an immune response which may complicate treatment using non-self blocking peptides.

The peptides of the invention may be introduced into APCs directly, e.g., by intravenous injection of a solution containing one or more of the peptides.

25 Alternatively, the APCs may be provided with a means of synthesizing large quantities of the blocking peptides intracellularly. Recombinant genes that encode ER and/or endosomal targeting signals fused to blocking peptide sequences are linked to appropriate expression control

30 sequences and introduced into APCs. Once in the cell, these genes direct the expression of the hybrid peptides. Peptides targeted to the ER will bind class II α and β chains as they are translated and assembled into heterodim rs. The presence of high affinity binding

35 peptides within the ER will prevent associati n of the

- 5 -

α/β complex with invariant chain, and thus interfere with intracellular trafficking. Th class II molecule/ blocking peptide complex may subsequently be expressed on the cell surface, but would not elicit an immune response

5 since T cells are tolerized to this complex early in development. The use of peptides tagged with ER retention signals may also prevent the peptide-complexed class II molecules from leaving the ER. Alternatively, the recombinant peptide may be tagged with an endosomal

10 targeting signal which directs it to the endosomal compartment after synthesis, thereby also skewing the ratio of endogenously-processed peptide to blocking peptide in the endosome and favoring binding of the high affinity blocking peptide to any class II molecules which

15 did not bind it in the ER. It may be advantageous, for any individual patient, to employ one or more ER-directed peptides in combination with one or more endosome-directed peptide, so that α-β complexes which are not filled in the ER with peptides of the invention are then

20 blocked in the endocytic pathway. The end result again is cell surface expression of a non-immunogenic class II/peptide complex.

        The use of a class II nonrestricted high affinity binding peptide coupled to an intracellular delivery

25 system permits the specific down-regulation of class II restricted immune responses without invoking the pleiotropic adverse reactions associated with the current pharmacological strategies. Successful application of these technologies will constitute a significant advance

30 towards the treatment of autoimmune disease and prevention of transplant rejection.

        The intracellular delivery system of the invention can also be utilized in a novel m thod of vaccination f an animal, e.g., a human patient or a commercially

35 significant mammal such as a cow which is susceptibl to

- 6 -

diseases such as hoof and mouth disease. Such a system
can be tailored to gen rate the type of immune response
required in a given situation by adjustments in the
following: (a) peptide specificity for class I or class
5 II MHC; (b) peptide/protein length and/or sequence, and
(c) using specific tags for organelle targeting. The
system of the invention ensures that peptides are
produced only within cells, and are not present outside
the cells where they could stimulate antibody production
10 by contact with B cells. This limits the immune response
generated by such a vaccine to T cell-mediated immunity,
thereby preventing either an inappropriate or potentially
deleterious response as might be observed with standard
vaccines targeting the organisms which cause, for
15 example, HIV, malaria, leprosy, and leishmaniasis.
Furthermore, this exclusively T cell-mediated immune
response can be class I or class II-based, or both,
depending upon the length and character of the
immunogenic peptides: MHC class I molecules are known to
20 bind preferentially to peptides 8 to 10 residues in
length, while class II molecules bind with high affinity
to peptides that range from 12 to 25 residues long.
      Immunization and therapy according to the
invention can employ a purified preparation of a peptide
25 of the invention, i.e., a peptide which includes an amino
acid sequence identical to that of a segment of a
naturally-occurring human protein (i.e., a "self
protein"), such segment being of 10 to 30 residues in
length, wherein the peptide binds to a human MHC class II
30 allotype, and preferably binds to at least two distinct
MHC class II allotypes (e.g., any of the approximately 70
known DR allotypes, approximately 47 known DP allotypes,
 r approximately 33 known DQ allotypes). Th portion of
the peptide corresponding to the self protein segment is
35 herein termed a "self peptide". By "purified

- 7 -

preparation" is meant a preparation at least 50% (by
w ight) of the polypeptide constituents of which consists
of the peptide of the invention. In preferred
embodiments, the peptide of the invention constitutes at

5 least 60% (more preferably at least 80%) of the purified
preparation. The naturally-occurring human protein is
preferably HLA-A2 (as broadly defined below), HLA-A29,
HLA-Bw62, HLA-C, HLA-DRα, HLA-DRβ, invariant chain (Ii),
Ig kappa chain C region, Ig heavy chain, Na$^+$/K$^+$ ATPase,

10 transferrin, transferrin receptor, calcitonin receptor,
carboxypeptidase E, MET kinase-related transforming
protein, guanylate-binding protein, mannose-binding
protein, apolipoprotein B-100, cathepsin C, cathepsin S,
metalloproteinase inhibitor 1 precursor, or heat shock

15 cognate 71 kD protein; it may be an MHC class I or II
antigen protein or any other human protein which occurs
at the cell surface of APCs. The self peptide preferably
conforms to the following motif: at a first reference
position (I) at or within 12 residues of the amino

20 terminal residue of the segment, a positively charged
residue (i.e., Lys, Arg, or His) or a large hydrophobic
residue (i.e., Phe, Trp, Leu, Ile, Met, Tyr, or Pro; and
at position I+5, a hydrogen bond donor residue (i.e.,
Tyr, Asn, Gln, Cys, Asp, Glu, Arg, Ser, Trp, or Thr). In

25 addition, the peptide may also be characterized as
having, at positions I+9, I+1, and/or I-1, a hydrophobic
residue (i.e., Phe, Trp, Leu, Ile, Met, Pro, Ala, Val, or
Tyr) (+ denotes positions to the right, or toward the
carboxy terminus,

30 and - denotes positions to the left, or toward the amino
terminus.) A typical peptide of the invention will
include a sequence corresponding to residues 31-40 (i.e.,
TQFVRFDSDA) or residues 106-115 (i.e., DWRFLRGYHQ) of
HLA-A2, or residues 107-116 (i.e., RMATPLLMQA) of Ii, or

- 8 -

a sequence essentially identical to any one of the
sequ nces set forth in Tabl s 1-10 below.

The therapeutic and immunization methods of the
invention can also employ a nucleic acid molecule (RNA or
5   DNA) encoding a peptide of the invention, but encoding
less than all of the entire sequence of the self protein.
The nucleic acid preferably encodes no substantial
portion of the self protein other than the specified self
peptide which binds to a MHC class II molecule, although
10   it may optionally include a signal peptide or other
trafficking sequence which was derived from the self
protein (or from another protein). A trafficking
sequence is an amino acid sequence which functions to
control intracellular trafficking (directed movement from
15   organelle to organelle or to the cell surface) of a
polypeptide to which it is attached. Such trafficking
sequences might traffic the polypeptide to ER, a
lysosome, or an endosome, and include signal peptides
(the amino terminal sequences which direct proteins into
20   the ER during translation), ER retention peptides such as
KDEL; and lysosome-targeting peptides such as KFERQ,
QREFK, and other pentapeptides having Q flanked on one
side by four residues selected from K, R, D, E, F, I, V,
and L. An example of a signal peptide that is useful in
25   the invention is a signal peptide substantially identical
to that of an MHC subunit such as class II α or β; e.g.,
the signal peptide of MHC class II α is contained in the
sequence MAISGVPVLGFFIIAVLMSAQESWA. The signal peptide
encoded by the nucleic acid of the invention may include
30   only a portion (e.g., at least ten amino acid residues)
of the specified 25 residue sequence, provided that
portion is sufficient to cause trafficking of the
polypeptid to the ER. In preferred embodiments, the
nucleic acid f the invention encodes a s cond self
35   peptide and a second trafficking sequenc (which may be

- 9 -

identical to or different than the first self peptid and
first trafficking sequenc), and it may ncode additional
self peptides and trafficking sequences as well.   In
still another variation on this aspect of the invention,
5 the self peptide sequence (or a plurality of self peptide
sequences arranged in tandem) is linked by a peptide bond
to a substantially intact Ii polypeptide, which then
carries the self peptide sequence along as it traffics
the class II molecule from ER to endosome.

10   The nucleic acid of the invention may also contain
expression control sequences (defined as transcription
and translation start signals, promoters, and enhancers
which permit and/or optimize expression of the coding
sequence with which they are associated) and/or genomic
15 nucleic acid of a phage or a virus, such as an attenuated
or non-replicative, non-virulent form of vaccinia virus,
adenovirus, Epstein-Barr virus, or a retrovirus.

          The peptides and nucleic acids of the invention
may be prepared for therapeutic use by suspending them
20 directly in a pharmaceutically acceptable carrier, or by
encapsulating them in liposomes, immune-stimulating
complexes (ISCOMS), or the like.   Such preparations are
useful for inhibiting an immune response in a human
patient, by contacting a plurality of the patient's APCs
25 with the therapeutic preparation and thereby introducing
the peptide or nucleic acid into the APCs.

          Also within the invention is a cell (e.g., a
tissue culture cell or a cell, such as a B cell or APC,
within a human) containing the nucleic acid molecule of
30 the invention. A cultured cell containing the nucleic
acid of the invention may be used to manufacture the
peptide of the invention, in a method which involves
culturing the cell under conditions permitting expression
of the peptide from the nucleic acid molecule.

- 10 -

Disclosed h rein is a method of identifying a nonallelically restricted immunomodulating p ptide, which method includes the steps of:

(a) fractionating a mixture of peptides eluted
5 from a first MHC class II allotype;

(b) identifying a self peptide from this mixture; and

(c) testing whether the self peptide binds to a second MHC class II allotype, such binding being an
10 indication that the self peptide is a nonallelically restricted immunomodulating peptide.

In further embodiments, the invention includes a method of identifying a potential immunomodulating peptide, in a method including the steps of:
15   (a) providing a cell expressing MHC class II molecules on its surface;

(b) introducing into the cell a nucleic acid encoding a candidate peptide; and

(c) determining whether the proportion of
20 class II molecules which are bound to the candidate peptide is increased in the presence of the nucleic acid compared to the proportion bound in the absence of the nucleic acid, such an increase being an indication that the candidate peptide is a potential immunomodulating
25 peptide.

Also within the invention is a method of identifying a potential immunomodulating peptide, which method includes the steps of:

(a) providing a cell expressing MHC class II
30 molecules on its surface;

(b) introducing into the cell a nucleic acid encoding a candidate peptide; and

(c) determining whether the level of MHC class II molecules on the surface of the cell is decreased in the
35 pr sence of the nucleic acid compared to the level of MHC

class II molecules in the absence of the nucleic acid,
such a decrease being an indication that the candidate
peptide is a potential immunomodulating peptide.

Also included in the invention is a method of
5 identifying a nonallelically restricted immunostimulating
peptide, which method includes the steps of:

(a) providing a cell bearing a first MHC class I
or class II allotype, such cell being infected with a
pathogen (e.g., an infective agent which causes human or
10 animal disease, such as human immunodeficiency virus
(HIV), hepatitis B virus, measles virus, rubella virus,
influenza virus, rabies virus, *Corynebacterium
diphtheriae, Bordetella pertussis, Plasmodium spp.,
Schistosoma spp., Leishmania spp., Trypanasoma spp.,* or
15 *Mycobacterium lepre*);

(b) eluting a mixture of peptides bound to the
cell's first MHC allotype;

(c) identifying a candidate peptide from the
mixture, such candidate peptide being a fragment of a
20 protein from the pathogen; and

(d) testing whether the candidate peptide binds
to a second MHC allotype, such binding being an
indication that the candidate peptide is a nonallelically
restricted immunostimulating peptide. A nucleic acid
25 encoding such an immunogenic fragment of a protein of a
pathogen can be used in a method of inducing an immune
response in a human patient, which method involves
introducing the nucleic acid into an APC of the patient.

The therapeutic methods of the invention solve
30 certain problems associated with prior art methods
involving intravenous injection of synthetic peptides:
(1) because of allelic specificity, a peptide capable of
binding with high affinity to all, or ven most, of the
diff rent class II allotypes expressed within the general

- 12 -

population had not previously b en identifi d; (2) the
half-lives of peptides delivered intravenously are
generally very low, necessitating repeated administration
with the associated high level of inconvenience and cost;
5   (3) this type of delivery approach requires that the
blocking peptide displace the naturally-occurring peptide
occupying the binding cleft of a class II molecule while
the latter is on the cell surface, which is now believed
to be a very inefficient process; and (4) if the blocking
10 peptide utilized is itself immunogenic, it may promote
deleterious immune responses in some patients.

        Other features and advantages of the invention
will be apparent from the following detailed description,
and from the claims.

15   Detailed Description

        The drawings are first briefly described.

Drawings

        Figs. 1a-f are chromatographic analyses of the
peptide pools extracted from papain digested HLA-DR1,
20 DR2, DR3, DR4, DR7, and DR8, respectively, illustrating
the peptide repertoire of each HLA-DR as detected by UV
absorbance. The UV absorbance for both 210 nm and 277 nm
is shown at a full scale absorbance of 500 mAU with a
retention window between 16 minutes and 90 minutes (each
25 mark represents 2 minutes).

        Fig. 2 is a representative mass spectrometric
analysis of the size distribution of isolated HLA-DR1
bound peptides. The determined peptide masses in groups
of 100 mass units were plotted against the number of
30 isolated peptides identified by mass spectrometry.
Peptide length was calculated by dividing the
experimental mass by an average amino acid mass of 118
daltons.

        Fig. 3 is a repres ntati n of two minigenes of the
35 invention, in which the HLA-DRα chain leader peptide is

- 13 -

linked to the amino terminus of a 15-residue (A) or 24-
residue (B) blocking peptide fragment of human invariant
chain Ii.

Experimental Data

5 METHODS

I. Purification of HLA-DR antigens.

HLA-DR molecules were purified from homozygous,
Epstein-Barr virus-transformed, human B lymphoblastoid
lines: DR1 from LG-2 cells, DR2 from MST cells, DR3 from
10 WT20 cells, DR4 from Priess cells, DR7 from Mann cells,
and DR8 from 23.1 cells. All of these cell lines are
publicly available. Cell growth, harvest conditions and
protein purification were as previously described (Gorga,
J. et al., 1991). Briefly, 200 grams of each cell type
15 was resuspended in 10mM Tris-HCl, 1mM dithiothreitol
(DTT), 0.1mM phenylmethylsulfonylflouride (PMSF), pH 8.0,
and lysed in a Thomas homogenizer. The nuclei were
removed by centrifugation at 4000xg for 5 min and the
pellets washed and repelleted until the supernatants were
20 clear. All the supernatants were pooled and the membrane
fraction harvested by centrifugation at 175,000xg for 40
min. The pellets were then resuspended in 10 mM Tris-
HCl, 1mM DTT, 1mM PMSF, 4% NP-40. The unsolubilized
membrane material was removed by centrifugation at
25 175,000xg for 2 hours, and the NP-40 soluble supernatant
fraction used in immunoaffinity purification.

Detergent soluble HLA-DR was bound to a LB3.1-
protein A sepharose column (Gorga et al., id) and eluted
with 100 mM glycine, pH 11.5. Following elution, the
30 sample was immediately neutralized by the addition of
Tris-HCl and then dialyzed against 10mM Tris-HCl, 0.1%
deoxycholic acid (DOC). The LB3.1 monoclonal antibody
recognizes a conformational determinant present on the

- 14 -

nonpolymorphic HLA-DRα chain, and thus recognizes all
all types of HLA-DR.

The transmembrane domain of the DR molecules was
removed by papain digestion, and the resulting water-
5   soluble molecule further purified by gel filtration
chromatography on an S-200 column equilibrated in 10mM
Tris-HCl, pH 8.0. The purified DR samples were
concentrated by ultrafiltration, yield determined by BCA
assay, and analyzed by SDS polyacrylamide gel
10 electrophoresis.

II. Extraction and fractionation of bound peptides.

Water-soluble, immunoaffinity-purified class II
molecules were further purified by high-performance size
exclusion chromatography (SEC), in 25 mM N-morpholino
15 ethane sulfonic acid (MES) pH 6.5 and a flowrate of
1 ml/min., to remove any residual small molecular weight
contaminants. Next, Centricon microconcentrators
(molecular weight cutoff 10,000 daltons) (Amicon Corp.)
were sequentially washed using SEC buffer and 10% acetic
20 acid prior to spin-concentration of the protein sample
(final volume between 100-200 μl). Peptide pools were
extracted from chosen class II alleles by the addition of
1 ml of 10% acetic acid for 15 minutes at 70°C. These
conditions are sufficient to free bound peptide from
25 class II molecules, yet mild enough to avoid peptide
degradation. The peptide pool was separated from the
class II molecule after centrifugation through the
Centricon concentrator, with the flow-through containing
the previously bound peptides.
30   The collected acid-extracted peptide pool was
concentrated in a Savant Speed-Vac to a volume of 50 μl
prior to HPLC separation. Peptides were separated on a
microbore C-18 reversed-phase chromatography (RPC) column
(Vydac) utilizing the following non-lin ar gradient
35 prot col at a constant flowrate of 0.15 ml/min.: 0-63

- 15 -

min. 5%-33% buffer B; 63-95 min. 33%-60% buffer B; 95-105
min 60%-80% buffer B, where buffer A was 0.06%
trifluoroacetic acid/water and buffer B was 0.055%
trifluoroacetic acid/acetonitrile. Chromatographic

5   analysis was monitored at multiple UV wavelengths (210,
254, 277, and 292 nm) simultaneously, permitting
spectrophotometric evaluation prior to mass and sequence
analyses. Shown in Fig.1 are chromatograms for each of
the six DR peptide pools analyzed. Collected fractions

10 were subsequently analyzed by mass spectrometry and Edman
sequencing.

III. Analysis of peptides.

     The spectrophotometric evaluation of the peptides
during RPC provides valuable information regarding amino

15 acid composition (contribution of aromatic amino acids)
and is used as a screening method for subsequent
characterization. Appropriate fractions collected during
the RPC separation were next analyzed using a Finnegan-
MAT LaserMat matrix-assisted laser-desorption mass

20 spectrometer (MALD-MS) to determine the individual mass
values for the predominant peptides. Between 1%-4% of
the collected fraction was mixed with matrix (1μl α-
Cyano-4-hydroxycinnamic acid) to achieve mass
determination of extracted peptides. The result of this

25 analysis for HLA-DR1 is shown in Fig. 2. Next, chosen
peptide samples were sequenced by automated Edman
degradation microsequencing using an ABI 477A protein
sequencer (Applied Biosystems) with carboxy-terminal
verification provided by mass spectral analysis using the

30 Finnigan-MAT TSQ 700 triple quadruple mass spectrometer
equipped with an electro-spray ion source. This parallel
analysis ensures complete identity of peptide composition
and sequence. Peptide alignment with protein sequences
stor d in the SWISS-PROT database was perf rmed using the

- 16 -

FASTA computer database search program. Set f rth in
Tabl s 1-10 are the r sults of this s qu nc analysis for
each of the DR molecules studied.

RESULTS

5 I. HLA-DR1.

        The HLA-DR1 used in this study was papain
solubilized to enable the material to be used both for
crystallographic and bound peptide analyses. The
peptides bound to DR1 were acid extracted and
10 fractionated using RPC (Fig. 1). The absence of any
detectable peptidic material following a second
extraction/RPC separation verified quantitative peptide
extraction. Amino acid analysis (ABI 420A/130A
derivatizer/HPLC) of extracted peptide pools demonstrated
15 a 70-80% yield, assuming total occupancy of purified DR1
with a molar equivalent of bound peptides corresponding
to the size distribution determined by mass spectrometry
(see Fig. 2). The RPC profiles obtained from DR1
extractions of multiple independent preparations were
20 reproducible. Furthermore, profiles from either
detergent-soluble or papain-solubilized DR1 were
equivalent. To confirm that the peptides were in fact
identical in detergent-soluble and papain-digested DR1,
mass spectrometry and Edman sequencing analyses were
25 performed and revealed identical masses and sequences for
analogous fractions from the two preparations.

        Matrix-assisted laser desorption mass spectrometry
(MALD-MS) was used to identify 111 species of unique mass
contained within the eluted peptide pool of DR1 with an
30 average size of 18 and a mode of 15 residues (Fig. 2).
Over 500 additional mass species present within the
molecular weight range of 13-25 residues were detected;
however, the signal was not sufficient to assign
individual masses with confidence. Multiple species of

- 17 -

varying mass were detected in fractions corresponding to
single RPC peaks indicating co-elution of peptides. To
characterize these peptides further, samples were
analyzed in parallel on a triple quadruple mass
5 spectrometer equipped with an electrospray ion source
(ESI-MS) and by automated Edman degradation
microsequencing (Lane et al., J. Prot. Chem. 10:151-160
(1991)). Combining these two techniques permits crucial
verification of both the N- and C-terminal amino acids of
10 peptides contained in single fractions. The sequence and
mass data acquired for twenty peptides isolated from DR1
are listed in Table 1. All the identified peptides
aligned with complete identity to regions of proteins
stored in the SWISS-PROT database.
15   Surprisingly, sixteen of the twenty sequenced DR1-
bound peptides were 100% identical to regions of the self
proteins HLA-A2 and class II-associated invariant chain
(Ii), representing at least 26% of the total extracted
peptide mass. These isolated peptides varied in length
20 and were truncated at both the N- and C-termini,
suggesting that: 1) antigen processing occurs from both
ends after binding to DR1, or 2) class II molecules bind
antigen from a pool of randomly generated peptides. The
yields from the peptide microsequencing indicated that
25 HLA-A2 (Fig. 1) and Ii each represents at least 13% of
the total DR1-bound peptides.
        An additional surprising finding concerned a
peptide which, although bound to HLA-DR and 100%
homologous with HLA-A2 peptide, was derived from a cell
30 which does not express HLA-A2 protein. Evidently this
peptide is derived from a protein containing a region
homologous with a region of HLA-A2 protein. Thus, for
purposes of this specificati n, the t rm "HLA-A2 protein"
is intended to include HLA-A2 protein itself, as well as
35 any naturally occurring protein which contains a ten or

- 18 -

great r amino acid long region of >80% homology with an
HLA-DR-binding peptide derived from HLA-A2. An "HLA-A2
peptide" similarly refers to peptides from any HLA-A2
protein, as broadly defined herein.

5   The other four peptides identified in the DR1
studies were derived from two self proteins, transferrin
receptor and the Na^+/K^+ ATPase, and one exogenous protein,
bovine serum fetuin (a protein present in the serum used
to fortify the medium which bathes the cells). Each of
10 these peptides occupied only 0.3-0.6% of the total DR1
population, significantly less than either the HLA-A2 or
the Ii peptides. It is known that class II molecules en
route to the cell surface intersect the pathway of
incoming endocytic vesicles. Both recycling membrane
15 proteins and endocytosed exogenous protein travel this
common pathway. Hence, the HLA-A2, transferrin receptor,
Na^+/K^+ ATPase and bovine fetuin derived peptides would all
encounter DR1 in a similar manner. Ii associates with
nascent class II molecules in the endoplasmic reticulum
20 (ER) (Jones et al., Mol. Immunol. 16:51-60 (1978)),
preventing antigen binding until the class II/Ii complex
arrives at an endocytic compartment (Roche and Cresswell,
Nature 345:615-618 (1990)), where Ii undergoes
proteolysis (Thomas et al., J. Immunol. 140:2670-2675
25 (1988); Roche and Cresswell, Proc. Natl. Acad. Sci. USA
88:3150-3154 (1991)), thus allowing peptide binding to
proceed. Presumably, the Ii peptides bound to DR1 were
generated at this step.
          Synthetic peptides corresponding to five of the
30 peptides reported in Table 1 were made and their relative
binding affinities to DR1 determined. The influenza A
hemagglutinin peptide (HA) 307-319 has been previously
described as a high affinity, HLA-DR1 restricted peptide
(Roche and Cresswell, J. Immunol. 144:1849-1856 (1990);
35 Rothbard et al., Cell 52:515-523 (1988)), and was thus

- 19 -

chosen as the control peptide. "Empty" DR1 purified from
ins ct cells expressing recombinant DR1 cDNA was used in
the binding experiments because of its higher binding
capacity and 10-fold faster association kinetics than DR1
5   isolated from human cells (Stern and Wiley, Cell 68:465-
477 (1992)). All the synthetic peptides were found to
compete well (Ki < 100 nM) against the HA peptide (Table
2). At first approximation, the Ii 106-119 peptide had
the highest affinity of all the competitor peptides
10 measured, equivalent to that determined for the control
HA peptide. In addition to the Ki determinations, these
peptides were found to confer resistance to SDS-induced
α-β chain dissociation of "empty" DR1 when analyzed by
SDS-PAGE, indicative of stable peptide binding (Sadegh-
15 Nasseri and Germain, Nature 353:167-170 (1991); Dornmair
et al., Cold Spring Harbor Symp. Quant. Biol. 54:409-415
(1989); Springer et al., J. Biol. Chem. 252:6201-6207
(1977)). Neither of the two control peptides, β_2m 52-64
nor Ii 96-110, was able to either confer resistance to
20 SDS-induced chain dissociation of DR1 or compete with HA
307-319 for binding to DR1; both of these peptides lack
the putative binding motif reported in this study (see
below).

A putative DR1 binding motif based on the sequence
25 alignments of the core epitopes (the minimum length) of
certain naturally processed peptides is shown in Table 3.
The peptides listed in this table include those
determined herein for HLA-DR1, as well as a number of
peptides identified by others and known to bind DR1
30 (reference #6 in this table being O'Sullivan et al., J.
Immunol. 145:1799-1808, 1990; reference #17, Roche &
Cresswell, J. Immunol. 144:1849-1856, 1990; reference
#25, Guttinger et al., Intern. Immunol. 3:899-906, 1991;
refer nce #27, Guttinger t al. EMBO J. 7:2555-2558,
35 1988; and refer nce #28, Harris et al., J. Immunol.

- 20 -

148:2169-2174, 1992). The key residues proposed in the
motif are as follows: a positively charged group is
located at the first position, referred to here as the
index position for orientation (I); a hydrogen bond donor
5 is located at I+5; and a hydrophobic residue is at I+9.
In addition, a hydrophobic residue is often found at I+1
and/or I-1. Every naturally processed peptide sequenced
from DR1 conforms to this motif (with the exception of
the HLA-A2 peptide 103-116 that lacks residue I+9).
10 Because the putative motif is not placed in a defined
position with respect to the first amino acid and because
of the irregular length of bound peptides, it is
impossible to deduce a motif from sequencing of peptide
pools, as was done for class I molecules (Falk et al.,
15 Nature 351:290-296 (1991)). The Ii 96-110 peptide, a
negative control peptide used in binding experiments, has
the I and I+5 motif residues within its sequence, but is
missing eight additional amino acids found in Ii 105-118
(Table 3C).
20   A sequence comparison of 35 previously described
DR1-binding synthetic peptides (O'Sullivan et al., J.
Immunol. 145:1799-1808 (1990); Guttinger et al., Intern.
Immunol. 3:899-906 (1991); Hill et al., J. Immunol.
147:189-197 (1991); Guttinger et al., EMBO J. 7:2555-2558
25 (1988); Harris et al., J. Immunol. 148:2169-2174 (1992))
also supports this motif. Of the 35 synthetic peptides,
21 (60%) have the precise motif, nine (30%) contain a
single shift at either I or I+9, and the remaining five
(10%) have a single substitution at I (Table 3B and C).
30 Interestingly, in the latter peptides, a positive charge
at I is always replaced by a large hydrophobic residue
(Table 8C); a pocket has been described in class I
molecules that can accommodate this precise substitution
(Latron et al., Proc. Natl. Acad. Sci. USA 88:11325-11329
35 (1991)). Contributions by the other eight amino acids

- 21 -

within the motif or the length of the peptide have not
been fully evaluated and may compensate for
shifted/missing residues in those peptides exhibiting
binding.   Evaluation of the remaining 117 non-DR1 binding
5 peptides cited in those studies (which peptides are not
included in Table 3) indicates that 99 (85%) of these
peptides do not contain the DR1 motif proposed herein.
Of the remaining 18 peptides (15%) that do not bind to
DR1 but which do contain the motif, 6 (5%) are known to
10 bind to other DR allotypes; the remaining 12 peptides may
have unfavorable interactions at other positions which
interfere with binding.

          In contrast to the precise N-terminal cleavages
observed in the previous study of six peptides bound to
15 the mouse class II antigen termed I-A^b and five bound to
mouse I-E^b (Rudensky et al., Nature 3563:622-627 (1991)),
the peptides bound to DR1 are heterogeneous at both the
N- and C-termini.   In contrast to peptides bound to class
I molecules, which are predominantly nonamers (Van Bleek
20 and Nathenson, Nature 348:213-216 (1990); Rotzschke et
al., Nature 348:252-254 (1990); Jardetzky et al., Nature
353:326-329 (1991); Hunt et al., Science 255:1261-1263
(1992)), class II peptides are larger and display a high
degree of heterogeneity both in length and the site of
25 terminal truncation, implying that the mechanisms of
processing for class I and class II peptides are
substantially different.   Furthermore, the present
results suggest that class II processing is a stochastic
event and that a DR allotype may bind peptides of
30 different lengths from a complex random mixture.   The
heterogeneity observed may be solely due to protection of
bound peptides from further degradation.   Thus, class II
molecules w uld play an active role in antigen processing
(as previously proposed (Donermeyer and Allen, J.
35 Immunol. 142:1063-1068 (1989)) by protecting the bound

- 22 -

peptides from complete degradation. Alternatively, the
predominance of 15mers bound to DR1 (as detected by both
the MALD-MS and the yields of sequenced peptides) could
be the result of trimming of bound peptides. In any
5 event, the absence of detectable amounts of peptides
shorter than 13 and longer than 25 residues suggests that
there are length constraints intrinsic either to the
mechanism of peptide binding or to antigen processing.
The predominance of peptides bound to DR1 that are
10 derived from endogenously synthesized proteins, and
particularly MHC-related proteins, may result from the
evolution of a mechanism for presentation of self
peptides in connection with the generation of self
tolerance.

15 **II. Other HLA-DR molecules.**

The sequences of naturally processed peptides
eluted from each of DR2, DR3, DR4, DR7 and DR8 are shown
in
Tables 4-8, respectively. Table 9 gives sequences of DR1
20 from another cell line which does not have wild-type Ar,
but which has bound A2-like peptides. Table 10 gives
sequences of peptides eluted from DR4 and DR11 molecules
expressed in cells from a human spleen. These data
demonstrate the great prevalence of self peptides bound,
25 compared to exogenous peptides. The data also show that
the A2 and Ii peptides occur repeatedly.

III. Peptide Delivery
Genetic Constructions.

In order to prepare genetic constructs for _in vivo_
30 administration of genes encoding immunomodulatory
peptides of the invention, the following proc dur is
carried out.

- 23 -

Overlapping synthetic oligonucleotides were used
to generate the leader peptid /blocking peptide mini-
genes illustrated in Fig. 3 by PCR amplification from
human HLA-DRα and invariant chain cDNA templates. These

5 mini-genes encode the Ii peptide fragments
KMRMATPLLMQALPM (or Ii_{15}) and LPKPPKPVSKMRMATPLLMQALPM (or
Ii_{24}). The resulting constructs were cloned into pGEM-2
(Promega Corp.) to form the plasmids pGEM-2-α-Ii_{15} and
pGEM-2-α-Ii_{24}, with an upstream T7 promoter for use in the

10 in vitro transcription/translation system described
below.

For in vivo expression, each mini-gene was
subsequently subcloned from the pGEM-2 derivatives into a
transfection vector, pHβactin-1-neo (Gunning et al.,

15 (1987) P.N.A.S. U.S.A. 84:4831), to form the plasmids
pHβactin-α-Ii_{15} and pHβactin-α-Ii_{24}. The inserted mini-
genes are thus expressed in vivo from the
constitutive/strong human β actin promoter. In addition,
the mini-genes were subcloned from the pGEM-2 derivatives

20 into the vaccinia virus recombination vector pSC11 (S.
Chakrabarti et al. (1985) Mol. Cell Biol. 5, 3403-3409)
to form the plasmids pSC11-α-Ii_{15} and pSC11-α-Ii_{24}.
Following recombination into the viral genome the
inserted mini-genes are expressed from the strong

25 vaccinia $p_{7.5}$ promoter.
Intracellular trafficking signals added to peptides.

Short amino acid sequences can act as signals to
target proteins to specific intracellular compartments.
For example, hydrophobic signal peptides are found at the

30 amino terminus of proteins destined for the ER, while the
sequence KFERQ (and other closely related sequences) is
known to target intracellular polypeptides to lysosomes,
while other sequences target polypeptides to endosomes.
In addition, th peptide sequence KDEL has b en shown to

35 act as a r tention signal for the ER. Each of these

- 24 -

signal peptides, or a combination thereof, can be used to
traffic the immunomodulating peptides of the invention as
desired. For example, a construct encoding a given
immunomodulating peptide linked to an ER-targeting signal
5 peptide would direct the peptide to the ER, where it
would bind to the class II molecule as it is assembled,
preventing the binding of intact Ii which is essential
for trafficking. Alternatively, a construct can be made
in which an ER retention signal on the peptide would help
10 prevent the class II molecule from ever leaving the ER.
If instead a peptide of the invention is targeted to the
endosomic compartment, this would ensure that large
quantities of the peptide are present when invariant
chain is replaced by processed peptides, thereby
15 increasing the likelihood that the peptide incorporated
into the class II complex is the high-affinity peptides
of the invention rather than naturally-occurring,
potentially immunogenic peptides. The likelihood of
peptides of the invention being available incorporation
20 into class II can be increased by linking the peptides to
an intact Ii polypeptide sequence. Since Ii is known to
traffic class II molecules to the endosomes, the hybrid
Ii would carry one or more copies of the peptide of the
invention along with the class II molecule; once in the
25 endosome, the hybrid Ii would be degraded by normal
endosomal processes to yield both multiple copies of the
peptide of the invention or molecules similar to it, and
an open class II binding cleft. DNAs encoding
immunomodulatory peptides containing targeting signals
30 will be generated by PCR or other standard genetic
engineering or synthetic techniques, and the ability of
these peptides to associate with DR molecules will be
analyzed *in vitro* and *in vivo*, as described bel w.

      It is proposed that the invariant chain prevents
35 class II molecules from binding peptides in the ER and

- 25 -

may contribute to heterodimer formation. Any mechanism
that prevents this association would increase th
effectiveness of class II blockade. Therefore, a peptide
corresponding to the site on Ii which binds to the class
5 II heterodimer, or corresponding to the site on either
the α or β subunit of the heterodimer which binds to Ii,
could be used to prevent this association and thereby
disrupt MHC class II function.

In Vitro Assembly.
10   Cell free extracts are used routinely for
expressing eukaryotic proteins (Krieg, P. & Melton, D.
(1984) Nucl. Acids Res. 12, 7057; Pelham, H. and Jackson,
R. (1976) Eur. J. Biochem. 67, 247). Specific mRNAs are
transcribed from DNA vectors containing viral RNA
15 polymerase promoters (Melton, D. et al. (1984) Nucl.
Acids Res. 12, 7035), and added to micrococcal nuclease-
treated cell extracts. The addition of ^{35}S methionine and
amino acids initiates translation of the exogenous mRNA,
resulting in labeled protein. Proteins may be
20 subsequently analyzed by SDS-PAGE and detected by
autoradiography. Processing events such as signal
peptide cleavage and core glycosylation are initiated by
the addition of microsomal vesicles during translation
(Walter, P. and Blobel, G. (1983), Meth. Enzymol., 96,
25 50), and these events are monitored by the altered
mobility of the proteins in SDS-PAGE gels.
        The ability of peptides containing a signal
peptide sequence to be accurately processed and to
compete with invariant chain for class II binding in the
30 ER are assayed in the *in vitro* system described above.
Specifically, DR1α and β chain, and invariant chain
peptide constructs described above ar transcribed into
mRNAs, which will b translated in th presence f
mammalian microsomal membranes. Ass ciation of the DR

- 26 -

h terodimer with Ii is det rmined by immunoprecipitation
with antisera to DR and Ii. Addition of mRNA encoding
the peptide of the invention to the translation reaction
should result in a decreased level of

5 coimmunoprecipitated Ii, and the concomitant appearance
of coimmunoprecipitated peptide, as determined by SDS-
PAGE on TRIS-Tricine gels. These experiments will
provide us with a rapid assay system for determining the
potential usefulness of a given blocking peptide as a

10 competitor for Ii chain binding in the ER. Those
peptides of the invention which prove to be capable of
competing successfully with Ii in this cell-free assay
can then be tested in intact cells, as described below.

In Vivo Assembly.

15   Human EBV-transformed B cell lines LG-2 and HOM-2
(homozygous for HLA-DR1) and the mouse B cell hybridoma
LK35.2 are transfected with either 50μg of linearized
pHβactin-α-Ii$_{15}$ or pHβactin-α-Ii$_{24}$ or (as a control)
pHβactin-1-neo by electroporation (150mV, 960μF, 0.2cm

20 cuvette gap). Following electroporation, the cells are
cultured in G418-free medium until total recovery
(approximately 4 days). Each population is then placed
under G418 selection until neomycin-expressing resistant
populations of transfectants are obtained (approximately

25 1-2 months). The resistant populations are subcloned by
limiting dilution and the clonality of stable
transfectants determined by PCR amplification of blocking
peptide mRNA expression.

        Stable transfectants of LG-2 and HOM-2 carrying
30 blocking peptide mini-genes or negative control vectors
are grown in large-scale culture conditions until 20
grams of pelleted cell mass is obtained. The HLA-DR
expressed by each transfectant is purified, and the bound
peptide repertoire   (both from within the cell and from

- 27 -

the cell surfac) analyzed as described above.
Successful demonstration of a reduction in the total
bound peptide diversity will be conclusive evidence of
intracellular delivery of immuno-modulatory peptides.

5   A second cell-based assay utilizes stable
transfectants of LK35.2 cells carrying blocking peptide
mini-genes or negative control vectors; these cells are
used as APCs in T cell proliferation assays. Each
transfectant is cultured for 24 hours in the presence of
10 different dilutions of hen egg lysozyme (HEL) and HEL-
specific T cell hybridomas. The relative activation of
the T cells present in each assay (as measured by
lymphokine production) is determined using the publicly
available lymphokine dependent cell line CTLL2 in a ^{3}H-
15 thymidine incorporation assay (Vignali et al. (1992)
J.E.M. 175:925-932). Successful demonstration of a
reduction in the ability of blocking peptide expressing
transfectants to present HEL to specific T cell
hybridomas will be conclusive evidence of intracellular
20 delivery of immuno-modulatory peptides. Cells of the
human TK^{-} cell line 143 (ATCC) are infected with vaccinia
virus (strain WR, TK^{+}) (ATCC), and two hours
postinfection, pSC11-α-Ii$_{15}$ or pSC11-α-Ii$_{24}$ or pSC11 is
introduced into the infected cells by calcium phosphate
25 precipitation. TK^{-} recombinants are selected for with
bromodeoxyuridine at 25μg/ml. Recombinant plaques are
screened by PCR for the presence of mini-gene DNA.
Recombinant virus is cloned by three rounds of limiting
dilution to generate pure clonal viral stocks.

30   In experiments analogous to the transfection
experiments described above, recombinant vaccinia viruses
encoding mini-genes or vector alone will be used to
infect large-scale cultures of the human EBV transformed
B cell lines LG-2 and HOM-2. Following infection, the
35 HLA-DR is purified and the bound peptid rep rtoire

- 28 -

analyzed as described above. A reduction of the
complexity of the bound peptide population and a
significant increase in the relative amount of Ii
peptides bound are conclusive evidence that vaccinia can
5 deliver blocking peptides to human APCs.

The same recombinant vaccinia viruses encoding
mini-genes or vector will be used to infect mice
experiencing experimentally-induced autoimmunity. A
number of such models are known and are referred in
10 Kronenberg, Cell 65:537-542 (1991).

*Liposomal Delivery of Synthetic Peptides or Mini-gene
Constructs.*

Liposomes have been successfully used as drug
carriers and more recently in safe and potent adjuvant
15 strategies for malaria vaccination in humans (Fries et
al. (1992), Proc. Natl. Acad. Sci. USA 89:358).
Encapsulated liposomes have been shown to incorporate
soluble proteins and deliver these antigens to cells for
both in vitro and in vivo CD8^{+} mediated CTL response
20 (Reddy et al., J. Immunol. 148:1585-1589, 1992; and
Collins et al., J. Immunol. 148:3336-3341, 1992). Thus,
liposomes may be used as a vehicle for delivering
synthetic peptides into APCs.

Harding et al. (Cell (1991) 64, 393-401) have
25 demonstrated that the targeting of liposome-delivered
antigen to either of two intracellular class II-loading
compartments, early endosomes and/or lysosomes, can be
accomplished by varying the membrane composition of the
liposome: acid-sensitive liposomes were found to target
their contents to early endosomes, while acid-resistant
30 liposomes were found to deliver their contents to
lysosomes. Thus, the peptides of the inv ntion will be
incorporated into acid-sensitive liposomes wher delivery

- 29 -

to endosomes is d sired, and into acid-resistant
liposomes for delivery to lysosomes.

Liposomes are prepared by standard detergent
dialysis or dehydration-rehydration methods. For acid-
5 sensitive liposomes, dioleoylphosphatidylethanolamine
(DOPE) and palmitoylhomocystein (PHC) are utilized, while
dioleoylphospatidylcholine (DOPC) and
dioleoylphosphatidylserine (DOPS) are used for the
preparation of acid-resistant liposomes. 10^{-5} mol of total
10 lipid (DOPC/DOPS or DOPE/PHC at 4:1 mol ratios) are
dried, hydrated in 0.2 ml of HEPES buffered saline (HBS)
(150 mM NaCl, 1 mM EGTA, 10mM HEPES pH 7.4) and
sonicated. The lipid suspensions are solubilized by the
addition of 0.1 ml of 1 M octylglucoside in HBS. The
15 peptides to be entrapped are added to 0.2 ml of 0.6 mM
peptide in 20% HBS. The mixture is then frozen,
lyophilized overnight, and rehydrated. These liposomes
will be treated with chymotrypsin to digest any surface-
bound peptide. Liposome delivery to EBV-transformed cell
20 lines (as described above) will be accomplished by 12-16
hour incubation at 37°C. HLA-DR will be purified from
the liposome treated cells and bound peptide analyzed as
above.

Alternatively, the liposomes are formulated with
25 the DNA mini-gene constructs of the invention, and used
to deliver the constructs into APCs either _in vitro_ or _in_
vivo.

Human immunization will be carried out under the
protocol approved by both The Johns Hopkins University
30 Joint Committee for Clinical Investigation and the Human
Subject Research Review Board of the Office of the
Surgeon General of the U.S. Army (Fries et al. (1992),
P.N.A.S. U.S.P. 89:358-362), using dosages described
therein, or other dosages described in the literature for
35 lipos me-based delivery of therapeutic agents.

- 30 -

Delivery via Immune-stimulating Complexes (ISCOMS).

    ISCOMS are negatively charged cage-like structures
of 30-40nm in size formed spontaneously on mixing
cholesterol and Quil A (saponin). Protective immunity
5 has been generated in a variety of experimental models of
infection, including toxoplasmosis and Epstein-Barr
virus-induced tumors, using ISCOMS as the delivery
vehicle for antigens (Mowat and Donachie) Immunology
Today 12:383-385, 1991. Doses of antigen as low as $1\mu g$
10 encapsulated in ISCOMS have been found to produce class I
mediated CTL responses, where either purified intact HIV-
1-IIIB gp 160 envelope glycoprotein or influenza
hemagglutinin is the antigen (Takahashi et al. , Nature
344:873-875, 1990). Peptides are delivered into tissue
15 culture cells using ISCOMS in a manner and dosage similar
to that described above for liposomes; the class II
peptide binding of delivered peptides are then determined
by extraction and characterization as described above.
ISCOM-delivered peptides of the invention which are
20 effectively utilized by cultured cells are then tested in
animals or humans.

    In addition to delivery of the therapeutic
synthetic peptides, ISCOMS could be constituted to
deliver the mini-gene constructs to APCs, and thus serve
25 as an alternative to the above-outlined vaccinia
strategy.

Immunogenic Peptide Delivery (Vaccines).

    In addition to using the above-described
intracellular delivery systems to deliver nonimmunogenic
30 self peptides with the specific aim of down-modulating
the immune system (thus alleviating autoimmune
conditions), the delivery systems of the invention may
alternatively be used as a novel means of vaccination, in
order t <u>stimulate</u> a portion of th immun syst m of an

- 31 -

animal. In the latter context, the deliv ry system is
empl yed to deliver, into appropriat cells, DNA
constructs which express immunogenic, pathogen-derived
peptides intended to stimulate an immune response against
5 a specific pathogen. Because the antigenic peptide is
produced inside the target cell itself, the vaccine
method of the invention ensures that there is no
circulating free antigen available to stimulate antibody
formation and thereby induce potentially deleterious or
10 inappropriate immunological reactions. The immune
response stimulated by vaccines of the invention is,
because the vaccines are targeted solely to APC's,
limited to the T cell mediated response, in contrast to
standard vaccine protocols which result in a more
15 generalized immune response. Although some of the
peptide-presenting APC's will initially be lysed by host
T cells, such lysis will be limited because, _inter alia_,
the virus-based vaccine is non-replicative, i.e., each
carrier virus can infect only one cell.
20   The model antigen that will be used to perfect and
test the system of the invention is hen egg lysozyme
(HEL). It is arguably the most well characterized
protein for antigen presentation studies, to which there
are numerous monoclonal antibodies and class I- and class
25 II-restricted mouse T cell clones and hybridomas. The
primary epitopes that will be studied are the peptide HEL
34-45, as both monoclonal antibodies and CD4+ T cell
hybridomas are available, and peptide HEL 46-61, as both
class I and class II-restricted T cell clones and
30 hybridomas have been raised and are publicly available.
These two sequences are thus proven immunogenic epitopes.
Initially, four constructs encoding different
polypeptides are analyzed: (a) whol , secreted HEL, (B)
HEL 34-45, (c) HEL 46-61, and (d) HEL 34-61. The last
35 three include a signal sequence known to be cleaved in

- 32 -

these cells, e.g., IA^k (MPRSRALILGVLALTTMLSLCGG), which
would result in targeting to the ER. All constructs are
then subcloned into pHβApr-1 neo. The methodology for
making these constructs is similar to that outlined

5   above. The constructs are introduced into appropriate
APCs, e.g., LK35.2 cells, by means of a conventional
eukaryotic transfection or one of the delivery vehicles
discussed above (e.g., vaccinia, liposomes, or ISCOMS).
LK35.2 cells, which possess the mouse MHC Class II

10 restriction molecules IA^k and IE^k, transfected with each
of the constructs are tested for their ability to
stimulate the appropriate class I and class II-restricted
T cell hybridomas and clones using standard techniques.
Whether class I stimulation is observed will depend on

15 whether peptide trimming can occur in the ER, in order to
produce an 8-10-mer suitable for binding to class I
molecules. If these constructs are ineffective for class
I stimulation, they can be modified in order to produce a
more effective peptide for class I binding. If these

20 constructs prove to be less effective for class II-
restricted responses, they can be tagged with endosomal
and/or lysosomal targeting sequences as discussed in
Section V.

        The effectiveness of targeting signals used to
25 direct immunogenic peptides to particular intracellular
organelles would be monitored using electron microscopic
analysis of immunogold stained sections of the various
transfectants. Rabbit anti-peptide antisera would be
produced and affinity purified for this application. In

30 addition, monoclonal antibody HF10, which recognizes HEL
34-45, will be used.

        Once a construct is defined that can be
effectively pres nt d by transfectants *in vitro*, its
effectiveness *in vivo* will be determined. This can b

35 tested by injection of the transfectants i.p. and/or s.c.

- 33 -

into C3H/Balb/c Fl mice, or by injection of the construct
incorporated into an appropriate delivery vehicle (e.g.,
liposome, ISCOMS, retrovirus, vaccinia). Optimal
protocols and doses for such immunizing injections can be
5   determined by one of ordinary skill in the art, given the
disclosures provided herein. Efficiency of immunization
can be tested by standard methods such as (a)
proliferation of class II-restricted T cells in response
to HEL pulsed APCs, (b) CTL response to ^{51}Cr-labeled
10   targets, and (c) serum antibody titre as determined by
ELISA.

     Once the details of the vaccine delivery system of
the invention are optimized, constructs encoding peptides
with useful immunizing potential can be incorporated into
15   the system. Such peptides can be identified by standard
means now used to identify immunogenic epitopes on
pathogen-derived proteins. For example, candidate
peptides for immunization may be determined from antibody
and T cell analysis of animals infected with a particular
20   pathogen. In order to obtain a protective and effective
anamnestic response, the peptides used for vaccination
should ideally be those which are presented with the
highest frequency and efficiency upon infection. This
could best be determined by using the procedures outlined
25   in the experimental section above to extract and
characterize the peptides bound by MHC class II molecules
from infected cells. Given allelic restriction of
immunogenic peptides (in contrast to the observed
degenerate binding of self peptides of invention), a
30   mini-gene encoding several immunogenic peptides will
probably be required to provide a vaccine useful for the
entire population. Vaccine administration and dosage are
as currently employed t smallpox vaccinati n.

          What is claimed is:

TABLE 1
LG-2/HLA-DR1 BINDING PEPTIDES

PROTEIN SOURCE	POSITION	SEQUENCE	LENGTH	FRACTION	MW	MASS SPEC	YIELD
HLA-A2	103-120	VGSDWRFLRGYHQYAYDG	18	DR1S-59	2190.4	2190.4	39.5
	103-117	VGSDWRFLRGYHQYA	15	DR1S-58	1855.0	1854.4	907.5
	103-116	VGSDWRFLRGYHQY	14	DR1S-58	1784.0	1783.6	53.3
	104-117	GSDWRFLRGYHQYA	14	DR1S-56	1755.3	1755.2	96.5
	105-117	SDWRFLRGYHQYA	13	DR1S-56	1698.2	1698.8	48.8
Invariant Chain (Ii)	96-120	LPKPPKPVSKMRMATPLLMQALPMG	25	DR1S-88	2733.5	2734.5	40.5
	96-119	LPKPPKPVSKMRMATPLLMQALPM	24	DR1S-88	2676.4	2675.9	80.8
	97-120	PKPPKPVSKMRMATPLLMQALPMG	24	DR1S-86	2620.2	2619.7	91.5
	96-118	LPKPPKPVSKMRMATPLLMQALP	23	DR1S-86	2545.2	2544.5	112.2
	97-119	PKPPKPVSKMRMATPLLMQALPM	23	DR1S-87	2563.2	2562.3	145.0
	98-119	KPPKPVSKMRMATPLLMQALPM	22	DR1S-87	2466.1	2465.8	101.5
	97-118	PKPPKPVSKMRMATPLLMQALP	22	DR1S-84	2432.0	2431.7	72.5
	98-118	KPPKPVSKMRMATPLLMQALP	21	DR1S-84	2334.9	2334.2	31.6
	98-118	PPKPVSKMRMATPLLMQALP	20	DR1S-86	2206.7	2207.4	89.8
	105-119	KMRMATPLLMQALPM	15	DR1S-88	1732.2	1731.9	178.5
	105-118	KMRMATPLLMQALP	14	DR1S-86	1601.0	1600.2	162.0
Na+/K+ ATPase	199-216	IPADLRIISANGCKVDNS	18	DR1S-56	1886.6	1885.8	48.8
Transferrin Recpt.	680-696	RVEYHFLSPYVSPKESP	17	DR1S-58	2035.3	2036.8	30.3
Bovine Fetuin	56-74	YKHTLNQIDSVKVWPRRPT	19	DR1S-51	2237.6	2236.5	69.0
	56-73	YKHTLNQIDSVKVWPRRP	18	DR1S-50	2338.7	2338.5	32.5
HLA-DR β-chain	43-61	DVGEYRAVTELGRPDAEYW	19	DR1S-51	2226.5	?	
Carboxypeptidase E	101-115	EPGEPEFKYIGNMHG	15	DR1S-48	1704.9	1700.4* ESI-MS	

TABLE 2

PEPTIDE BINDING TO HLA-DR1

PEPTIDE[a]	LENGTH	Ki vs HA 307-319[b] nM	SDS-Resistance[c] nM
HLA-A2 103-117	15	49 ± 3	+
II 105-119	15	< 10	+
II 96-119	24	33 ± 5	+
Na+/K+ ATPase 199-216	18	68 ± 9	+
Transf. Recept. 680-696	17	< 10	+
Bovine Fetuin 56-72	19	66 ± 18	
HA 307-319	14	< 10	+
II 96-110	15	> 10^4	-
β_2m 52-64	13	> 10^4	-

a   The first six entries correspond to peptides found associated with HLA-DR1 and the sequences are shown in Table 1. Two control peptides were also tested: β_2m 52-64, SDLSFSKDWSFYL, is from human β_2-microglobulin and II 96-110, LPKPPKPVSKMRMAT is a truncated version of the longest invariant chain derived peptide isolated from HLA-DR1. Peptides were synthesized using solid-phase Fmoc chemistry, deprotected and cleaved using standard methods, then purified by RPC. Purified peptides were analyzed by mass spectrometry and concentrations were determined by quantitative ninhydrin analysis.

b   Inhibition constants (Ki) were measured as the concentration of test peptide which inhibited 50% of the ^{125}I-labeled HA 307-319 binding to "empty" HLA-DR1 produced in Sf9 insect cells (20). HA 307-319 was labeled using Na^{125}I and chloramine-T and isolated by gel filtration. Specific activity, determined by BCA assay (Pierce) and gamma counting, was 26,000 cpm/pmol. 10nM labeled peptide and 10 nM purified HLA-DR1 were mixed with 10 different concentrations (10 nM to 10 µM) of synthetic cold competitor peptide in phosphate-buffered saline, pH 7.2, containing 1 mM EDTA, 1mM PMSF, 0.1 mM iodoacetamide, and 3 mM NaN$_3$, and incubated at 37°C for 85 hours. Free and bound peptide were separated by native gel electrophoresis (33) and bound radioactivity was quantitated using a Fujix imaging plate analyzer (BAS 2000) after four hour exposures on the phospho-imaging plates. Percent inhibition was calculated as the ratio of background-corrected radioactivity in the sample to background-corrected radioactivity in a parallel sample containing no competitor peptide. Under these conditions, Ki measurements < 10 nM could not be accurately determined.

c   The ability of the synthetic peptides to confer resistance to SDS-induced chain dissociation of HLA-DR1 produced in insect cells was determined as described (20). Briefly, 20 µM HLA-DR1 was incubated with five-fold excess of synthetic peptide at 37°C for 85 hours, in phosphate-buffered saline (pH 7.2) with the protease inhibitor mixture described above. After incubation, the samples were analyzed by SDS-PAGE with and without boiling prior to loading. Peptides which prevented SDS-induced chain dissociation are indicated positive (+) and those that did not negative (-).

TABLE 3 - PUTATIVE HLA-DR1 PEPTIDE BINDING MOTIF

	PROTEIN SOURCE	PEPTIDE SEQUENCE	LENGTH	POSITION	REFERENCE
A	HLA-A2	SDWRFLRGYHQYA	13	105-117	This study
	Invariant Chain	KMRMATPLLMQALP	14	105-118	6
	Na+/K+ ATPase	IPADLRIISANGCKYDNS	18	199-216	6
	Transferrin Receptor	RVEYHFLSPYVSPKESP	17	680-696	6
	Bovine Fetuin	YKHTLMQIDSVKVWPRRP	18	56-73	6
B	HEL	KVFGRCELAAAMKRHGLD	18	1-18	6
		RNRCKGTDVQAWIRGCRL	18	112-129	6
		HPPHIEIQMLKNGKKI	16	31-46	6
	β₂m	NELGRFKHTDACGRTH	16	19-34	6
	PLA₂	SKRVYQWFQLRKY	14	115-128	6
	NASE	ATSTKKLHKEPATLIKAIDG	20	1-20	6
		PATLIKAIDGQTVKLMYKGQ	20	11-30	6
		DRVKLMYKGQPMTFRLLLVD	20	21-40	6
		VAYVYKPWNTHEQHLRKSEA	20	111-130	6
	HIV p13	QKGEPIQKELYPLTSL	16	97-112	6
	HIV p17	GARASVLSGGELDKWE	16	1-16	6
	Influenza HA	RTLYQNVGTYVSVGTSTLNK	20	187-206	6
	Influenza HA	PKYVKQNTLKLAT	13	307-319	17
	P. falcip. p190	LKKLVFGYRKPLDNI	15	249-263	25
	P. falcip. CS	KNIEQYLKKIKNS	13	329-341	27
	Chicken OVA	DVFKELKVHHANENIF	16	15-30	6
	DR1 β chain	GDTRPRFLWQLKFECHFFNG	20	1-20	28
		TERVRLLEGCIYWQEESVRFDS	22	21-42	28
		DLLEGRRAAVDTYCRHNYGVGESFT	25	66-90	28
	p Cyt c	KAERAQLIAYLKQATAK	17	88-104	6
	Myelin basic prot.	GRTQDENPVVHFFKNIVTPRTPPP	24	75-98	6
C	Influenza Matrix	PLKAEIAQRLEDV	13	19-31	6
	HIV p17	RQILGQLQPSLQTGSE	16	57-72	6
	β₂M	IQVYSRHPPEHGKPHI	16	7-22	6
	PLA₂	INTCYKLEHPVTGCG	16	85-100	6

Table 3, continued

A PROTEIN SOURCE	PEPTIDE SEQUENCE	LENGTH	POSITION	REFERENCE
P. falcip. p190	YKLNFYFDLLRAKL	14	211-224	25
	IDTLKKNEHIKEL	13	338-350	25
DR1 β chain	DVGEYRAVTELGRPDAEYWN	20	43-62	28
HIV p17	ERFAVNPGLLETSEGC	16	41-56	6
HEL	DNYRGYSLGNWVCAAKFESNFTQ	23	20-42	6
NASE	EALVRQGLAKVAYVYKPNNT	20	101-120	6
HIV p25	PIVQNLQGQMVHQAIS	16	1-16	6
	SALSEGATPODLNTML	16	41-56	6
β₂m	SFYILAHTEFTPTETD	16	61-76	6
PLA₂	KMYFNLINTCYKLEH	16	79-94	6

TABLE 4

MST/HLA-DR2 BINDING PEPTIES

PROTEIN SOURCE	POSITION	SEQUENCE	LENGTH	FRACTION	MW	MASS SPEC
Pseudo HLA-A2	103-120	VGSDWRFLRGYHQYAYDG	18	DR2-3-57	2190.6	2189.0
	103-119	VGSDWRFLRGYHQYAYAD	17	DR2-3-57	2133.3	2131.8
	104-120	GSDWRFLRGYHQYAYDG	17	DR2-3-56	2034.3	2040.4
	103-117	VGSDWRFLRGYHQYA	15	DR2-3-56	1855.0	1858.5
	103-116	VGSDWRFLRGYHQY	14	DR2-3-56	1784.0	1786.3
	103-116	GSDWRFLRGYHQYA	14	DR2-3-55	1755.3	1755.0*
	104-117	GSDWRFLRGYHQYA	14	DR2-3-56	1698.2	1702.6
	105-117	SDWRFLRGYHQYA	13	DR2-3-56	2676.4	2675.0*
Invariant Chain	96-119	LPKPPKPVSKMRMATPLLMQALPM	24	DR2-3-70	2563.2	2562.0*
(ii)	97-119	PKPPKPVSKMRMATPLLMQALPM	23	DR2-3-70	2466.1	2465.0*
	98-119	KPPKPVSKMRMATPLLMQALPM	22	DR2-3-70	2432.0	2437.0
	97-118	PKPPKPVSKMRMATPLLMQALP	22	DR2-3-66	2334.9	2340.0
	98-118	KPPKPVSKMRMATPLLMQALP	21	DR2-3-66	2206.7	2207.0*
	99-118	PPKPVSKMRMATPLLMQALP	20	DR2-3-70	2070.5	2074.3
	105-123	KMRMATPLLMQALPMGALP	19	DR2-3-71	1752.2	1732.0*
	105-119	KMRMATPLLMQALPM	15	DR2-3-70	2746.1	2746.6
	59-81	EHHIFLGATNYIYVLNEEDLQKV	23	DR2-3-65		
(MET) Kinase-relate Trasforming protein	434-450	QELKHKYYQVPRKGIQA	17	DR2-3-71	2063.6	2074.3
Guanylate-bind.	174-193	IQNLIKEEAFLGITDEKTEG	20	DR2-3-70	2248.5	2248.0*
Mannose-bind. prot.	1-127	GDTRPRFLGQDKYECHFFNGTERVRFL	127	DR2-3-65	15055	15097
	1-126	GDTRPRFLGQDKYECHFFNGTERVRFL	126	DR2-3-66	14941	15013
HLA-DR2a β-chain	1-127	GDTRPRFLWQPKRECHFFNGTERVRFL	127	DR2-3-70		?
HLA-DR2b β-chain	1-126	GDTRPRFLWQPKRECHFFNGTERVRFL	126	DR2-3-71	15009	15009
	94-111	RVQPKVTVYPSKTQPLQH	18	DR2-3-39	2106.5	2114.
	94-108	RVQPKVTVYPSKTQP	15	DR2-3-39	1728.3	1730.6

ESI-MS*

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TABLE 5
UT-20/HLA-DR3 NATURALLY PROCESSED PEPTIDES

Protein Source	Position	Sequence	Length	Fraction	MW	Mass Spec.
Pseudo HLA-A2	103-117	VGSDWRFLRGYHQYA	15	DR3-2-63	1855.0	1863.9
Apolipoprotein B-100 (Human)	1276-1295	NFLKSDGRIKYTLNKNSLK	20	DR3-2-63	2352.9	2360.0
	1273-1291	IPDNLFLKSDGRIKYTLNK	19	DR3-2-63	2235.5	2245.1
	1276-1291	NLFLKSDGRIKYTLNK	16	DR3-2-60	1910.2	1911.4
	1276-1290	NLFLKSDGRIKYTLN	15	DR3-2-60	1782.1	1785.9
	1207-1224	YANILLDRRVPQTDMTF	17	DR3-2-63	2053.3	2059.1
HLA-DR β-chain	1-18	GDTRPRFLEYSTSECHFF	18	DR3-2-73	?	?
Invariant chain (Ii)	96-118	LPKPPKPVSKMRMATPLLMQALP	23	DR3-2-73	2545.2	2554.0
	97-118	PKPPKPVSKMRMATPLLMQALP	22	DR3-2-73	2432.0	2441.4
	98-118	KPPKPVSKMRMATPLLMQALP	21	DR3-2-73	2334.9	2345.3
						MALD-MS

TABLE 6

PRIESS/HLA-DR4 NATURALLY PROCESSED PEPTIDES

PROTEIN SOURCE	POSITION	SEQUENCE	LENGTH	FRACTION	MW	MASS SPEC
Ig Kappa Chain C region (Human)	188-208	KHKVYACEVTHQGLSSPVTKS	21	DR4-2-45	2299.6	2304.0
	188-207	KHKVYACEVTHQGLSSPVTK	20	DR4-2-47	2212.5	2213.0
	189-206	HKVYACEVTHQGLSSPVT	18	DR4-2-43	1955.5	1952.1
	188-204	KHKVYACEVTHQGLSSP	17	DR4-2-45	1883.1	1882.8
	187-203	EKHKVYACEVTHQGLSS	17	DR4-2-45	1915.1	1922.5
	188-203	KHKVYACEVTHQGLSS	16	DR4-2-54	1787.0	1787.0
	189-204	HKVYACEVTHQGLSSP	16	DR4-2-47	1755.0	1767.8
	187-202	EKHKVYACEVTHQGLS	16	DR4-2-43	1828.0	1822.8
	188-202	KHKVYACEVTHQGLS	15	DR4-2-51	1699.9	1708.3
	189-203	HKVYACEVTHQGLSS	15	DR4-2-45	1657.8	1657.0
	187-200	EKHKVYACEVTHQG	14	DR4-2-51	1628.8	1632.6
HLA-DR α-chain	182-198	APSPLPETTENVVCALG	17	DR4-2-43	1697.9	1700
HLA-A2	28-48	VDDTQFVRFDSDAASQRMEPR	21	DR4-2-56	2470.6	2472.9
	28-47	VDDTQFVRFDSDAASQRMEP	20	DR4-2-59	2314.5	2319.3
	28-46	VDDTQFVRFDSDAASQRME	19	DR4-2-54	2217.2	2218.7
	30-48	DTQFVRFDSDAASQRMEPR	19	DR4-2-55	2256.4	2263.2
	31-49	TQFVRFDSDAASQRMEPRA	19	DR4-2-56	2212.4	2211.5
	28-44	VDDTQFVRFDSDAASQR	17	DR4-2-55	1957.0	1963.1
	31-47	TQFVRFDSDAASQRMEP	17	DR4-2-56	1985.1	1987.5
	31-45	TQFVRFDSDAASQRM	15	DR4-2-54	1758.9	1761.0
	31-42	TQFVRFDSDAAS	12	DR4-2-54	1343.4	1343.3

Table 6, continued

PROTEIN SOURCE	POSITION	SEQUENCE	LENGTH	FRACTION	MW	MASS SPEC
HLA-C	28-50	VDDTQFVRFDSDAASPRGEPRAP	23	DR4-2-56	2533.7	2536.7
	31-52	TQFVRFDSDAASPRGEPRAPW	22	DR4-2-54	2489.7	2491.5
	28-48	VDDTQFVRFDSDAASPRGEPR	21	DR4-2-54	2365.5	2368.1
	28-47	VDDTQFVRFDSDAASPRGEP	20	DR4-2-56	2209.3	2211.5
	28-46	VDDTQFVRFDSDAASPRGE	19	DR4-2-56	2112.2	2113.9
	28-45	VDDTQFVRFDSDAASPRG	18	DR4-2-55	1983.1	1987.5
	31-48	TQFVRFDSDAASPRGEPR	18	DR4-2-52	2036.2	2041.5
	28-44	VDDTQFVRFDSDAASPR	17	DR4-2-55	1926.0	1931.7
	30-46	DTQFVRFDSDAASPRGE	17	DR4-2-52	1897.9	1901.6
	31-44	TQFVRFDSDAASPR	14	DR4-2-52	1596.7	1603.7
	31-42	TQFVRFDSDAAS	12	DR4-2-54	1343.4	1343.3
HLA-C	130-150	LRSWTAADTAAQITQRKWEAA	21	DR4-2-56	2374.6	2376.4
	129-145	DLRSWTAADTAAQITQR	17	DR4-2-59	1904.5	1908.7
	129-144	DLRSWTAADTAAQITQ	16	DR4-2-59	1747.9	1752.3
	129-143	DLRSWTAADTAAQIT	15	DR4-2-59	1619.7	1622.2
HLA-Bw62	129-145	DLSSWTAADTAAQITQR	17	DR4-2-60	1834.9	1838.1
	129-148	DLSSWTAADTAAQITQRKWE	20	DR4-2-66	2278.4	2284.6
Cathepsin C (Rat Homologue)	151-167	YDHNFVKAINADQKSWT	17	DR4-2-70	2037.2 / 2035.3	2039.6
	151-166	YDHNFVKAINADQKSW	16	DR4-2-70	1936.1 / 1934.2	1937.7
HLA-DR β-chain	1-16	GDTRPRFLEQVKHE	14	DR4-2-72	1711.9	
IG Heavy Chain	121-?	GVFFYLQWGRSTLVSVS (?)	(?)	DR4-2-6	?	?

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TABLE 7

MANN/HLA-DR7 NATURALLY PROCESSED PEPTIDES

PROTEIN SOURCE	POSITION	SEQUENCE	LENGTH	FRACTION	MW	MASS SPEC
Pseudo HLA-A2	103-120	VGSDMRFLRGYHQYAYDG	18	DR7-2-63	2190.4	2194
	103-117	VGSDMRFLRGYHQYA	15	DR7-2-63	1855.0	1860
HLA-A29	234-253	RPAGDGTFQKWASVVVPSGQ	20	DR7-2-66	2087.3	2092
	234-249	RPAGDGTFQKWASVVV	16	DR7-2-63	1717	1718
	237-258	GDGTFQKWASVVVPSGQEGRYT	22	DR7-2-66	2436	2440
	237-254	GDGTFQKWASVVVPSGQE	18	DR7-2-66	1892.3	1892
	239-252	GTFQKWASVVVPSG	14	DR7-2-66	1462	1465
	239-253	GTFQKWASVVVPSGQ	15	DR7-2-66	1718	1721
	239-261	GTFQKWASVVVPSGQEGRYTCHV	23	DR7-2-66	2603	2606
HLA-DR α-chain	58-78	GALANIAVDKANLEIMKRSH	21	DR7-2-66	2229.5	2221
Heat shock cognate 71 kD protein	38-54	TPSYVAFTDTERLIGDA	17	DR7-2-69	1856.0	1856.6
	38-54	TPSYVAFTDTERLIGDA	17	DR7-2-72	1856.0	1857.0
	38-52	TPSYVAFTDTERLIG	15	DR7-2-69	1669.8	1671.9
Invariant chain (Ii)	97-118	PKPPKPVSKMRMATPLLMQALP	22	DR7-2-72	2432.0	2436.6
	98-118	KPPKPVSKMRMATPLLMQALP	21	DR7-2-72	2334.9	2339.7
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TABLE 8
23.1/HLA-DR8 NATURALLY PROCESSED PEPTIDES

PROTEIN SOURCE	POSITION	SEQUENCE	LENGTH	FRACTION	MW	MASS SPEC
Metalloproteinase Inhibitor 1 Prec. (Human)	102-117	RSEEFLIAGKLQDGLL	16	DR8-3-70	1789.0	1799.9
	101-117	SEEFLIAGKLQDGLL	15	DR8-3-72	1632.9	1646.0
Transferrin (Bov?)	261-275	DVIWELLNHAQEHFG	15	DR8-3-78	1808.0	1818.1
Calcitonin receptor (Hum?)	38-53	EPFLYILGKSRVLEAQ	16	DR8-3-78	1863.2	1848.4
Cathepsin S	189-203	TAFQYIIDNKGIDSD	15	DR8-3-63	1699.8	1711.0
	189-202	TAFQYIIDNKGIDS	14	DR8-3-63	1584.7	1595.0

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TABLE 9

MHC2/HLA-DR1 NATURALLY PROCESSED PEPTIDES

PROTEIN SOURCE	POSITION	SEQUENCE	LENGTH	FRACTION	MW	MASS SPEC
Pseudo HLA-A2	103-117	VGSDWRFLRGYHQYA	15	M2/DR1-1-64	1855.0	1854.4
	104-117	GSDWRFLRGYHQYA	14	M2/DR1-1-63	1755.3	1755.2
Invariant Chain	96-119	LPKPPKPVSKMRMATPLLMQALPM	24	M2/DR1-1-77	2676.4	2675.9
(Ii)	97-120	PKPPKPVSKMRMATPLLMQALPMG	24	M2/DR1-1-72	2620.2	2619.7
	96-118	LPKPPKPVSKMRMATPLLMQALP	23	M2/DR1-1-73	2545.2	2544.5
	97-119	PKPPKPVSKMRMATPLLMQALPM	23	M2/DR1-1-75	2563.2	2562.3
	98-119	KPPKPVSKMRMATPLLMQALPM	22	M2/DR1-1-75	2466.1	2465.8
	97-118	PKPPKPVSKMRMATPLLMQALP	22	M2/DR1-1-72	2432.0	2431.7
	98-118	KPPKPVSKMRMATPLLMQALP	21	M2/DR1-1-72	2334.9	2334.2
						ESI-MS

TABLE 10

HUMAN SPLEEN DR4/DR11 NATURALLY PROCESSED PEPTIDES

PROTEIN SOURCE	POSITION	SEQUENCE	LENGTH	FRACTION	MW	MASS SPEC
HLA-DR α-chain	133-156	SETVFLPREDHLFRKFHYLPFLPS	24	FFR.391-71	2976.4	2982.5
	136-156	VFLPREDHLFRKFHYLPFLPS	21	FFR.391-71	2659.1	2665.9
	136-155	VFLPREDHLFRKFHYLPFLP	20	FFR.391-71	2572.0	2579.6
	136-151	VFLPREDHLFRKFHYL	16	FFR.391-71	2117.5	2126.6
Calgranulin B	25-50	KLGHPDTLNQGEFKELVRKDLQNFLK	26	FFR.391-71	3068.5	3073.0
	25-48	KLGHPDTLNQGEFKELVRKDLQNF	24	FFR.391-71	2827.2	2831.8
	25-38	KLGHPDTLNQGEFK	14	FFR.391-71	1583.8	1591.2

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- 47 -

CLAIMS

1. A purified preparati n of a p ptide comprising
an amino acid sequence identical to that of a segment of
a naturally-occurring human protein, said segment being
5 of 10 to 30 residues in length, inclusive, wherein said
peptide binds to a human major histocompatibility complex
(MHC) class II allotype.

2. The preparation of claim 1, wherein said
peptide binds to at least two distinct MHC class II
10 allotypes.

3. The preparation of claim 1, wherein said human
protein is HLA-A2, HLA-A29, HLA-Bw62, HLA-C, HLA-DRα,
HLA-DRβ, invariant chain (Ii), Ig kappa chain C region,
Ig heavy chain, Na⁺/K⁺ ATPase, transferrin, transferrin
15 receptor, calcitonin receptor, carboxypeptidase E, MET
kinase-related transforming protein, guanylate-binding
protein, mannose-binding protein, apolipoprotein B-100,
cathepsin C, cathepsin S, metalloproteinase inhibitor 1
precursor, or heat shock cognate 71 kD protein.

20   4. The preparation of claim 1, wherein said human
protein is an MHC class I or II molecule.

5. The preparation of claim 1, wherein said
segment conforms to the following motif:
          at a first reference position (I) at or within 12
25 residues of the amino terminal residue of said segment, a
positively charged residue or a large hydrophobic
residue; and
          at position I+5, a hydrogen bond donor residue.

6. Th preparation of claim 5, wher in said motif
30 c mprises a hydrophobic residue at I+9.

- 48 -

7.   The preparation of claim 6, wher in said motif
additionally comprises, at position I+1 or I-1, a
hydrophobic residue.

8.   The preparation of claim 1, wherein said
5 segment comprises residues 29-40 or residues 106-115 of
HLA-A2.

9.   The preparation of claim 1, wherein said
segment comprises residues 107-116 of Ii.

10.   A therapeutic composition comprising
10   (a) a peptide comprising an amino acid sequence
identical to that of a segment of a naturally-occurring
human protein, said segment being of 10 to 30 residues in
length, wherein said peptide binds to a human major
histocompatibility complex (MHC) class II allotype; and
15   (b) a pharmaceutically acceptable carrier.

11.   A liposome containing a peptide comprising an
amino acid sequence identical to that of a segment of a
naturally-occurring human protein, said segment being of
10 to 30 residues in length, wherein said peptide binds
20 to a human major histocompatibility complex (MHC) class
II allotype.

12.   An immune-stimulating complex (ISCOM)
comprising a peptide comprising an amino acid sequence
identical to that of a segment of a naturally-occurring
25 human protein, said segment being of 10 to 30 residues in
length, wherein said peptide binds to a human major
histocompatibility complex (MHC) class II allotype.

13.   A meth d of inhibiting an immune response in
a human patient, which method compris s contacting an

- 49 -

antigen-presenting cell (APC) of a patient with the
therapeutic composition of claim 10.

     14.   A method of inhibiting an immune response in
a human patient, which method comprises contacting an APC
5 of a patient with the liposome of claim 11.

     15. A method of inhibiting an immune response in
a human patient, which method comprises contacting an APC
of a patient with the ISCOM of claim 12.

     16.   A nucleic acid encoding a polypeptide, said
10 polypeptide comprising a first and a second amino acid
sequence linked by a peptide bond, said first sequence
being identical to that of a segment of a naturally-
occurring human protein, which segment binds to a human
MHC class II allotype and is of 10 to 30 residues in
15 length; and said second sequence being a sequence which
controls intracellular trafficking of a polypeptide to
which it is attached ("trafficking sequence").

     17.   The nucleic acid of claim 16, wherein said
trafficking sequence traffics said polypeptide to
20 endoplasmic reticulum (ER), a lysosome, or an endosome.

     18.   The nucleic acid of claim 16, wherein said
second sequence is substantially identical to the signal
peptide of an MHC subunit.

     19.   The nucleic acid of claim 18, wherein said
25 subunit is an MHC class II α or β subunit.

     20.   The nucleic acid of claim 16, wherein said
trafficking sequence is KDEL; KFERQ; QREFK;
MAISGVPVLGFFIIAVLMSAQESWA; a p ntapeptide comprising Q

- 50 -

flanked on one side by four residues selected from K, R,
D, E, F, I, V, and L; or a signal peptide.

21.   A liposome or ISCOM comprising the nucleic
acid of claim 16.

5   22.   A nucleic acid encoding a polypeptide
comprising a first and a second amino acid sequence
linked by a peptide bond, said first sequence being
identical to that of a segment of a naturally-occurring
human protein, which segment binds to a human MHC class
10   II allotype and is of 10 to 30 residues in length; and
said second sequence being substantially identical to
human Ii.

23.   The nucleic acid of claim 22, wherein said
polypeptide comprises a plurality of copies of said first
15   sequence linked in tandem to said second sequence.

24.   A nucleic acid molecule encoding a self
peptide comprising an amino acid sequence identical to
that of a segment of a naturally-occurring human protein,
said segment being of 10 to 30 residues in length,
20   wherein said self peptide binds to a human major
histocompatibility complex (MHC) class II allotype, and
wherein said nucleic acid molecule encodes less than the
entire sequence of said protein.

25.   The nucleic acid molecule of claim 24,
25   wherein said molecule additionally encodes a peptide
sequence which controls intracellular trafficking of a
polypeptide to which it is attached ("trafficking
sequence").

- 51 -

26.   The nucleic acid molecul of claim 25,
wherein said molecule additionally encodes a second self
peptide and a second trafficking sequence.

27.   The nucleic acid molecule of claim 24,
5 wherein said molecule additionally comprises expression
control elements.

28.   The nucleic acid molecule of claim 24,
wherein said molecule comprises plasmid or viral genomic
sequence.

10   29.   The nucleic acid molecule of claim 28,
wherein said molecule is the genome of a non-replicative,
non-virulent vaccinia virus, adenovirus, Epstein-Barr
virus, or retrovirus.

30.   A liposome or ISCOM comprising the nucleic
15 acid molecule of claim 24.

31.   A cell comprising the nucleic acid molecule
of claim 27.

32.   The cell of claim 31, wherein said cell is a
human B cell or APC.

20   33.   The cell of claim 31, wherein said nucleic
acid comprises genomic nucleic acid of a virus.

34.   A method of making a peptide, which method
comprises culturing the cell of claim 31 under conditions
permitting expression of said peptide from said nucleic
25 acid molecule.

- 52 -

35. A method of inhibiting an immun response in a human patient, which method comprises introducing the nucleic acid of claim 24 into a plurality of APCs of said patient.

5   36. A therapeutic composition comprising the nucleic acid of claim 24 in a pharmaceutically acceptable carrier.

37. A method of inducing an immune response in a human patient, which method comprises introducing into an 10 APC of said patient a nucleic acid molecule encoding an immunogenic fragment of a protein of other than human origin, wherein said fragment binds to an MHC class I or II molecule.

38. The method of claim 37, wherein said protein 15 is of an infective agent which causes human or animal disease.

39. The method of claim 38, wherein said infective agent is human immunodeficiency virus (HIV), hepatitis B virus, measles virus, rubella virus, 20 influenza virus, rabies virus, Corynebacterium diphtheriae, Bordetella pertussis, Plasmodium spp., Schistosoma spp., Leishmania spp., Trypanasoma spp., or Mycobacterium lepre.

40. The preparation of claim 1, wherein said 25 segment consists essentially of a sequence set forth in any of Tables 1-10.

41. A method of identifying a nonallelically restrict d immun modulating peptid , said method comprising:

- 53 -

      (a)   fractionating a mixture of peptides eluted from a first MHC class II allotype;

      (b)   identifying a self peptide from said mixture;

      (c)   testing whether said self peptide binds to a second MHC class II allotype, said binding being an indication that said self peptide is a nonallelically restricted immunomodulating peptide.

42. A method of identifying a potential immunomodulating peptide, said method comprising:

      (a)   providing a cell expressing MHC class II molecules on its surface;

      (b)   introducing into said cell a nucleic acid encoding a candidate peptide;

      (c)   determining whether the proportion of said class II molecules which are bound to said candidate peptide is increased in the presence of said nucleic acid compared to the proportion bound in the absence of said nucleic acid, said increase being an indication that said candidate peptide is a potential immunomodulating peptide.

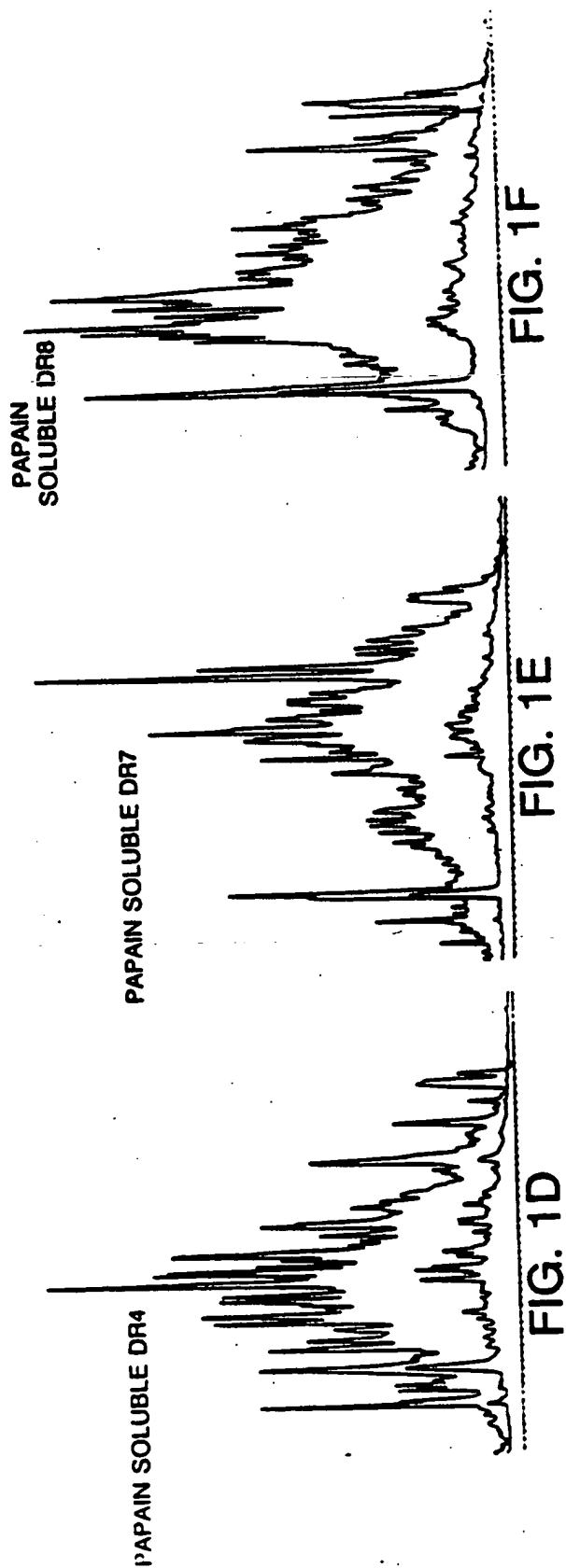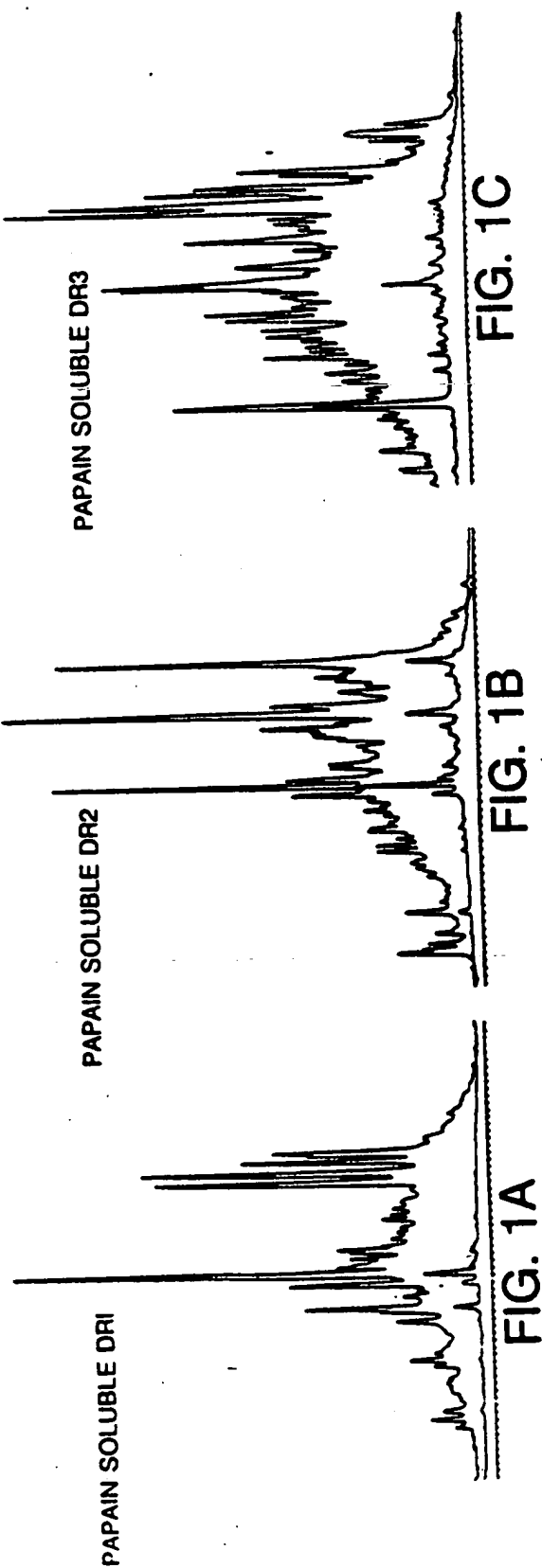43. A method of identifying a potential immunomodulating peptide, said method comprising:

      (a)   providing a cell expressing MHC class II molecules on its surface;

      (b)   introducing into said cell a nucleic acid encoding a candidate peptide;

      (c)   determining whether the level of MHC class II molecules on the surface of said cell is decreased in the presence of said nucleic acid compared to the level of said molecules in the absence of said nucleic acid, said decrease being an indication that said candidate peptide is a potential immunomodulating peptide.

- 54 -

44.   A method of identifying a nonallelically
restricted immunostimulating peptide, said meth d
comprising:
        (a)   providing a cell bearing a first MHC class I
5   or class II allotype, said cell being infected with a
pathogen;
        (b)   eluting a mixture of peptides bound to said
cell's first MHC allotype;
        (c)   identifying a candidate peptide from said
10   mixture, said candidate peptide being a fragment of a
protein from said pathogen;
        (d)   testing whether said candidate peptide binds
to a second MHC allotype, said binding being an
indication that said candidate peptide is a
15   nonallelically restricted immunostimulating peptide.
APPL3376

PAPAIN SOLUBLE DR3

FIG. 1C

PAPAIN
SOLUBLE DR8

FIG. 1F

PAPAIN SOLUBLE DR2

FIG. 1B

PAPAIN SOLUBLE DR7

FIG. 1E

PAPAIN SOLUBLE DRI

FIG. 1A

PAPAIN SOLUBLE DR4

FIG. 1D
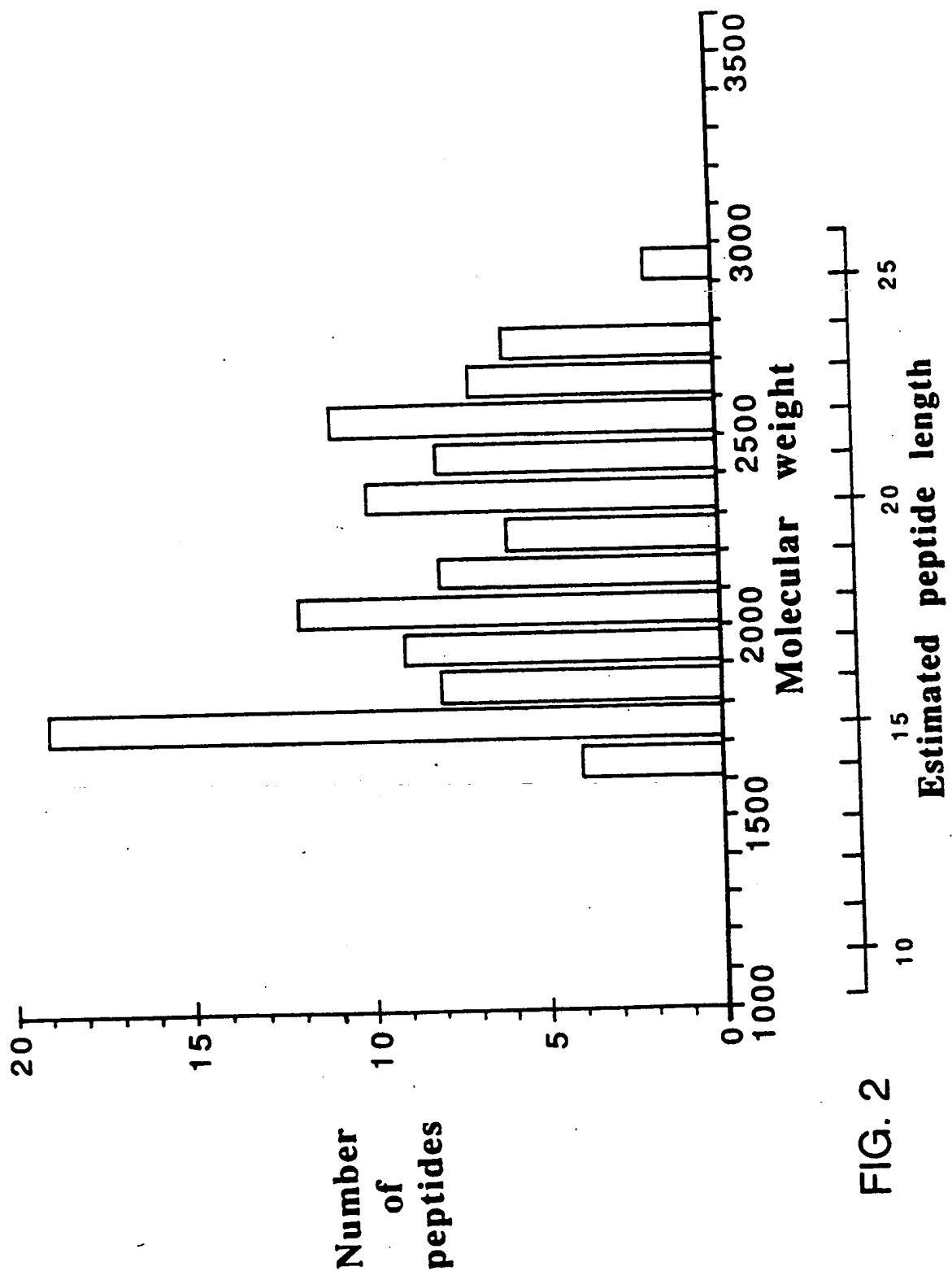
FIG. 2

ATG GCC ATA AGT GGA GTC CCT GTG CTA GGA TTT TTC ATC ATA GCT
M   A   I   S   G   V   P   V   L   G   F   F   I   I   A

GTG CTG ATG AGC GCT CAG GAA TCA TGG GCT AAG ATG CGC ATG GCC
V   L   M   S   A   Q   E   S   W   A   K   M   R   M   A

ACC CCG CTG CTG ATG CAG GCG CTG CCC ATG TAA
T   P   L   L   M   Q   A   L   P   M   stop

FIG. 3A

ATG GCC ATA AGT GGA GTC CCT GTG CTA GGA TTT TTC ATC ATA GCT
M   A   I   S   G   V   P   V   L   G   F   F   I   I   A

GTG CTG ATG AGC GCT CAG GAA TCA TGG GCT CTT CCC AAG CCT CCC
V   L   M   S   A   Q   E   S   W   A   L   P   K   P   P

AAG CCT GTG AGC AAG ATG CGC ATG GCC ACC CCG CTG CTG ATG CAG
K   P   V   S   K   M   R   M   A   T   P   L   L   M   Q

GCG CTG CCC ATG TAA
A   L   P   M   stop

FIG. 3B

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 39/00, 39/385, 37/22; C07K 7/00; C12N 5/12
US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/88, 450;
530/300; 536/27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US, A, 5,130,297 (Sharma, et al.) 14 July 1992. See entire document.	1,2,10,11-15 1-44
A	US, A, 4,681,760 (Fathman) 21 July 1987, see entire document.	1-44
A	Critical reviews in Immunology, vol. 11(5), issued 1992, J.C. Gorga, "Structural analysis of class II major histocompatibility complex proteins," pages 305-335, see entire document.	1-44
A	US, A, 4,478,823 (Sanderson) 23 October 1984. See entire document.	1-44
A	Immunol. Res., vol. 6, issued 1987, D.H. Margulies et al. "Engineering soluble major histocompatibility molecules: why and how", pages 101-116, see entire article.	1-44
A	NATURE, vol. 348, issued 13 December 1990, F.M. Brodsky, "The invariant dating service", pages 581-582.	1-44

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	
"E"	earlier document published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O"	document referring to an oral disclosure, use, exhibition or other means	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"P"	document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 SEPTEMBER 1992	30 SEP 1992

Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer THOMAS M. CUNNINGHAM
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-0196

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